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L25	L24 AND equilibrium gradient	5	L25
L24	L23 AND density gradient	280	L24
L23	L22 AND equilibrium	918	L23
L22	L21 AND density	4961	L22
L21	L20 AND gradient	7337	L21
L20	vesicle	19333	L20
L19	Berg-Eric-A.IN.	5	L19
L18	Fine-Richard-E.IN.	2	L18
L17	dense core vesicles	54	L17
<i>DB=USPT,PGPB; PLUR=YES; OP=ADJ</i>			
L16	L14 AND vesicle	35	L16
L15	L14 AND microsome	2	L15
L14	L13 AND sucrose gradient	206	L14
L13	((435/7.1  435/317.1 )!.CCLS. )	6316	L13
<i>DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ</i>			
L12	L11 AND equilibrium gradient	32	L12
L11	sucrose gradient	2910	L11
<i>DB=USPT,PGPB; PLUR=YES; OP=ADJ</i>			
L10	L9 AND equilibrium density gradient	1	L10
L9	L8 AND sucrose gradient	265	L9
L8	((435/325  435/352  435/366  435/368 )!.CCLS. )	11207	L8
L7	L6 AND equilibrium density gradient	4	L7
L6	L3 AND sucrose gradient	482	L6
L5	L3 AND sucrose velocity size gradient	0	L5
L4	L3 sucrose velocity size gradient	0	L4
L3	((530/300  530/350 )!.CCLS. )	11943	L3
<i>DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ</i>			
L2	Berg-Eric.IN.	1	L2
L1	(Fine-Richard.IN.)	4	L1

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<u>L14</u>	dense core vesicles	55	<u>L14</u>
<u>L13</u>	sucrose equilibrium density gradient	7	<u>L13</u>
<u>L12</u>	L11 AND equilibrium	75	<u>L12</u>
<u>L11</u>	L10 AND vesicle	361	<u>L11</u>
<u>L10</u>	sucrose gradient	2917	<u>L10</u>
<u>L9</u>	L8 AND velocity	61	<u>L9</u>
<u>L8</u>	L7 AND equilibrium	402	<u>L8</u>
<u>L7</u>	sucrose gradient	2917	<u>L7</u>
<u>L6</u>	L5 AND sucrose gradient	5	<u>L6</u>
<u>L5</u>	microsome preparation	85	<u>L5</u>
<u>L4</u>	sucrose equilibrium density gradient	7	<u>L4</u>
<u>L3</u>	sucrose velocity size gradient	1	<u>L3</u>
<u>L2</u>	velocity size AND equilibrium density	1	<u>L2</u>
<u>L1</u>	(velocity size gradient AND equilibrium density gradient)	0	<u>L1</u>

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BRAIN; ANALYZING DENSE CORE VESICLES FROM BRAIN TISSUES; O~~IN~~ IN BRAIN  
CELLS, INCUBATE WITH EXTRACTION BUFFER, MONITOR VESICLE TISSUES  
IN Berg Eric A; Fine Richard E  
PA Boston University (1308)  
PI US 2002090655 A1 20020711  
AI US 2001-1898 20011031  
PRAI US 2000-244971P 20001101 (Provisional)  
FI US 2002090655 20020711  
DT Utility; Patent Application - First Publication  
FS CHEMICAL  
CLMN APPLICATION  
CLMN 15  
GI 6 Figure(s).

FIGS. 1A and 1B disclose the separation of substance P containing vesicles in a sucrose velocity gradient. Preparations of microsomes from ON and LGN/SC respectively, were centrifuged in a 10-50% sucrose velocity gradient and analyzed for substance P by RIA. A, Substance P distribution of fractionated ON microsomes; and B, Substance P distribution of fractionated LGN/SC microsomes by radioimmunoassay.

FIGS. 2A-2E disclose the fractionation of substance P-containing vesicles in a sucrose equilibrium gradient. Fractions containing substance P from FIG. 1 were pooled, concentrated, and then loaded on an equilibrium density sucrose gradient (25%)%. Fractions were analyzed for A, substance P content (ON); B, substance P content (LGN/SC) by RIA; C, individual synaptic vesicle membrane proteins (SV2, synaptotagmin, and synaptophysin) (ON); D, individual synaptic vesicle membrane proteins (SV2, synaptotagmin, synaptotagmin, synaptophysin, and synaptobrevin) (LGN/SC); E, synaptotagmin IV (LGN/SC) by Western blot.

FIGS. 3A-3E disclose co-sedimentation of vesicle-associated proteins with substance P in LGN/SC microsomes fractionated by size and density. Fractions containing substance P from FIG. 2 were analyzed for A, secretogranin II; B, beta APP (C8); C, Rab3, D, alpha-synuclein; E, BDNF by Western blot.

FIG. 4 discloses immunoadsorption of synaptic vesicle membrane proteins from substance P-containing fractions. Fractions containing substance P from FIG. 2 were immunoabsorbed with mouse IgG or synaptophysin Ab linked magnetic beads (2 or 4 mgs) (Dynal) as per the manufacturers instructions. Samples were analyzed for specific synaptic vesicle membrane proteins (SV2, synaptotagmin, synaptophysin, and synaptobrevin) by Western blot.

FIGS. 5A and 5B disclose electron micrographs of an immunolabeled, negatively stained DCV preparation from LGN/SC. Fractions containing substance P from an equilibrium gradient were fixed, adhered to formvar, carbon coated nickel grids and A, immunolabeled with mouse IgG and colloidal gold conjugated antibodies (12 nm); or B, immunolabeled with synaptophysin and colloidal gold conjugated antibodies (12 nm).

FIGS. 6A-6D disclose electron micrographs of immunolabeled thin sections from a DCV preparation from LGN/SC. Fractions containing substance P from an equilibrium gradient were fixed, embedded, sectioned and immunolabeled with A, rabbit IgG and colloidal gold conjugated synaptotagmin and colloidal gold conjugated antibodies (12 nm); and D, BDNF and colloidal gold conjugated antibodies (6 nm).

=> s velocity size gradient AND equilibrium density gradient

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=> s size gradient AND equilibrium gradient AND vesicle

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L6 3 SIZE GRADIENT AND EQUILIBRIUM GRADIENT AND VESICLE

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IN Berg Eric A; Fine Richard E  
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AN 2002-435699 [46] WPIDS

DNN N2002-342949 DNC C2002-123804

TI Method for analyzing purified dense core \*\*\*vesicles\*\*\*, comprises determining contents of purified dense core \*\*\*vesicles\*\*\* from extracted brain samples which are purified with predetermined fold.

DC B04 S03

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PI WO 2002037104 A2 20020510 (200246)\* EN 33p G01N033-50

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